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## ABSTRACT

The invention relates to genetically engineered soluble fusion proteins consisting of human proteins or parts thereof not belonging to the immunoglobulin family and various portions of the constant region of immunoglobulin molecules. The functional properties of both fusion components are surprisingly retained in the fusion protein.

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COMPLETE SPECIFICATION

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FUSION PROTEINS WITH IMMUNOGLOBULIN PORTIONS, THE PREPARATIONS AND  
USE THEREOF

SENT TO PUBLIC INSPECTION  
UNDER  
SECTION 60 AND RULE 117  
JNL NO. 1672 OF 11172

APPLICATION NO. 2256/91  
SPECIFICATION NO. 23641

BEHRINGWERKE AKTIENGESELLSCHAFT, a Joint Stock Company organized an  
existing under the laws of the Federal Republic of Germany, of  
D-3550 Marburg, Federal Republic of Germany, and THE GENERAL  
HOSPITAL CORPORATION, a corporation organised under the laws of the  
State of Massachusetts, United States of America, of Fruit Street,  
Boston, Massachusetts 02114, United States of America.

Behringwerke Aktiengesellschaft  
and  
The General Hospital Corporation

NOE 90/B 026 - Ma 824  
r. LP

#### Description

5 Fusion proteins with immunoglobulin portions, the preparation and use thereof

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10 The invention relates to genetically engineered soluble fusion proteins composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of the constant region of immunoglobulin molecules. The functional properties of the two fusion partners are, surprisingly, retained in the fusion protein.

15 EP-A 0 325 262 and EP-A 0 314 317 disclose corresponding fusion proteins composed of various domains of the CD4 membrane protein of human T cells and of human IgG1 portions. Some of these fusion proteins bind with the same affinity to the glycoprotein gp120 of human immuno-  
20 deficiency virus as the cell-bound CD4 molecule. The CD4 molecule belongs to the immunoglobulin family and, consequently, has a very similar tertiary structure to that of immunoglobulin molecules. This also applies to the  $\alpha$  chain of the T-cell antigen receptor, for which such fusions have also been described (Gascoigne et al.,  
25 Proc. Natl. Acad. Sci. USA, vol. 84 (1987), 2937-2940). Hence, on the basis of the very similar domain structure, in this case retention of the biological activity of the two fusion partners in the fusion protein was to be expected.

30 The human proteins which are, according to the invention, preferably coupled to the amino terminus of the constant region of immunoglobulin do not belong to the immuno-  
globulin family and are to be assigned to the following classes: (i) membrane-bound proteins whose extracellular  
35 domain is wholly or partly incorporated in the fusion. These are, in particular, thromboplastin and cytokine

receptors and growth factor receptors, such as the cellular receptors for interleukin-4, interleukin-7, tumor necrosis factor, GM-CSF, G-CSF, erythropoietin; (ii) non-membrane-bound soluble proteins which are wholly or partly incorporated in the fusion. These are, in particular, proteins of therapeutic interest such as, for example, erythropoietin and other cytokines and growth factors.

The fusion proteins can be prepared in known pro- and eukaryotic expression systems, but preferably in mammalian cells (for example CHO, COS and BHK cells).

The fusion proteins according to the invention are, by reason of their immunoglobulin portion, easy to purify by affinity chromatography and have improved pharmacokinetic properties in vivo.

In many cases, the Fc part in fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations.

There are in existence various proteases whose use for this purpose appears conceivable. Papain and pepsin are employed, for example, to generate F(ab) fragments from immunoglobulins (Immunology, ed. Roitt, I. et al., Gower Medical Publishing, London (1989)), but they do not cleave in a particularly specific manner. Blood coagulation factor Xa by contrast recognises in a protein the relatively rare tetrapeptide sequence Ile-Glu-Gly-Arg and performs a hydrolytic cleavage of the protein after the

arginine residue. Sequences which contain the described tetrapeptide were introduced first by Nagai and Thogersen in a hybrid protein by genetic engineering means (Nagai, K. and Thogersen, H.C., Nature, vol. 309 (1984), 810-812). These authors were able to show that the proteins expressed in E. coli actually are specifically cleaved by factor Xa. However, there is as yet no published example of the possibility of such proteins also being expressed in eukaryotic and, especially, in animal cells and, after their purification, being cleaved by factor Xa. However, expression of the proteins according to the invention in animal cells is preferable because only in a cell system of this type is there expected to be secretion of, for example, normally membrane-bound receptors as fusion partners with retention of their natural structure and thus of their biological activity. Secretion into the cell culture supernatant facilitates the subsequent straightforward purification of the fusion protein.

The invention thus relates to genetically engineered soluble fusion proteins composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as immunoglobulin is the constant part of the heavy chain of human IgG, particularly preferably of human IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed in a simple way by a cleavage sequence which is also incorporated and can be cleaved with factor Xa.

Furthermore, the invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for diagnosis and therapy.

Finally, the invention is explained in further examples.

**Example 1: Thromboplastin fusion proteins**

Blood coagulation is a process of central importance in the human body. There is appropriately delicate regulation of the coagulation cascade, in which a large number of cellular factors and plasma proteins cooperate. These proteins (and their cofactors) in their entirety are called coagulation factors. The final products of the coagulation cascade are thrombin, which induces the aggregation of blood platelets, and fibrin which stabilizes the platelet thrombus. Thrombin catalyzes the formation of fibrin from fibrinogen and itself is formed by limited proteolysis of prothrombin. Activated factor X (factor Xa) is responsible for this step and, in the presence of factor Va and calcium ions, binds to platelet membranes and cleaves prothrombin.

Two ways exist for factor X to be activated, the extrinsic and the intrinsic pathway. In the intrinsic pathway a series of factors is activated by proteolysis in order for each of them to form active proteases. In the extrinsic pathway, there is increased synthesis of thromboplastin (tissue factor) by damaged cells, and it activates factor X, together with factor VIIa and calcium ions. It was formerly assumed that the activity of thromboplastin is confined to this reaction. However, the thromboplastin/VIIa complex also intervenes to activate the intrinsic pathway at the level of factor IX. Thus, a thromboplastin/VIIa complex is one of the most important physiological activators of blood coagulation.

It is therefore conceivable that thromboplastin, apart from its use as diagnostic aid (see below), can also be employed as constituent of therapeutic agents for treating inborn or acquired blood coagulation deficiencies. Examples of this are chronic hemophilias caused by a deficiency of factors VIII, IX or XI or else acute disturbances of blood coagulation as a consequence of, for example, liver or kidney disease. Use of such a

therapeutic agent after surgical intervention would also be conceivable.

Thromboplastin is an integral membrane protein which does not belong to the immunoglobulin family. Thromboplastin cDNA sequences have been published by a total of four groups (Fisher et al., *Thromb. Res.*, vol. 48 (1987), 89-99; Morrissey et al., *Cell*, vol. 50 (1987), 129-135; Scarpati et al., *Biochemistry*, vol. 26 (1987), 5234-5238; Spicer et al., *Proc. Natl. Acad. Sci. USA*, vol. 84 (1987), 5148-5152). Thromboplastin cDNA contains an open reading frame which codes for a polypeptide of 295 amino-acid residues, of which the 32 N-terminal amino acids act as signal peptide. Mature thromboplastin comprises 263 amino-acid residues and has a three-domain structure: i) amino-terminal extracellular domain (219 amino-acid residues); ii) transmembrane region (23 amino-acid residues); iii) cytoplasmic domain (carboxyl terminus; 21 amino-acid residues). In the extracellular domain there are three potential sites for N-glycosylation (Asn-X-Thr). Thromboplastin is normally glycosylated but glycosylation does not appear essential for the activity of the protein (Paborsky et al., *Biochemistry*, vol. 29 (1989), 8072-8077).

Thromboplastin is required as additive to plasma samples in diagnostic tests of coagulation. The coagulation status of the tested person can be found by the one-stage prothrombin clotting time determination (for example Quick's test). The thromboplastin required for diagnostic tests is currently obtained from human tissue, and the preparation process is difficult to standardize, the yield is low and considerable amounts of human starting material (placentae) must be supplied. On the other hand, it is to be expected that preparation of native, membrane-bound thromboplastin by genetic engineering will also be difficult owing to complex purification processes. These difficulties can be avoided by the fusion according to the invention to immunoglobulin portions.

The thromboplastin fusion proteins according to the invention are secreted by mammalian cells (for example CHO, BHK, COS cells) into the culture medium, purified by affinity chromatography on protein A-Sepharose and have surprisingly high activity in the one-stage prothrombin clotting time determination.

#### Cloning of thromboplastin cDNA

The sequence published by Scarpati et al., Biochemistry, vol. 26 (1987), 5234-5238, was used for cloning the thromboplastin cDNA. Two oligonucleotide probe molecules (see Fig. 1) were derived from this. These two probe molecules were used to screen a cDNA bank from human placenta (Grundmann et al., Proc. Natl. Acad. Sci. USA, vol. 83 (1986), 8024-8028).

cDNA clones of various lengths were obtained. One clone, 2b-Apr5, which is used for the subsequent procedure, codes for the same amino-acid sequence as the cDNA described in Scarpati et al. Fig. 2 depicts the total sequence of the clone 2b-Apr5 with the thromboplastin amino-acid sequence deduced therefrom.

#### Construction of a hybrid plasmid pTF1Fc coding for thromboplastin fusion protein.

The plasmid pCD4E gamma 1 (EP 0 325 262 A2; deposited at the ATCC under the number No. 67610) is used for expression of a fusion protein composed of human CD4 receptor and human IgG1. The DNA sequence coding for the extracellular domain of CD4 is deleted from this plasmid using the restriction enzymes HindIII and BamHI. Only partial cleavage must be carried out with the enzyme HindIII in this case, in order to cut at only one of the two HindIII sites contained in pCD4E gamma 1 (position 2198). The result is an opened vector in which a eukaryotic transcription regulation sequence (promoter) is followed by the open HindIII site. The open BamHI site is



located at the start of the coding regions for a pentapeptide linker, followed by the hinge and the CH2 and CH3 domains of human IgG1. The reading frame in the BamHI recognition sequence GGATCC is such that GAT is translated as aspartic acid. DNA amplification with thermostable DNA polymerase makes it possible to modify a given sequence in such a way that any desired sequences are attached at one or both ends. Two oligonucleotides able to hybridize with sequences in the 5'-untranslated region (A: 5' GATCGATTAAGCTTCGGAACCCGCTCGATCTCGCCGCC 3') or coding region (B: 5' GCATATCTGGATCCCCGTAGAATATTTCTCTGAATTCCCC 3') of thromboplastin cDNA were synthesized. Of these, oligonucleotide A is partially homologous with the sequence of the coding strand, and oligonucleotide B is partially homologous with the non-coding strand; cf. Fig. 3.

Thus, amplification results in a DNA fragment (827 bp) which contains (based on the coding strand) at the 5' end before the start of the coding sequence a HindIII site, and at the 3' end after the codon for the first three amino-acid residues of the transmembrane region a BamHI site. The reading frame in the BamHI cleavage site is such that ligation with the BamHI site in pCD4E gamma 1 results in a gene fusion with a reading frame continuous from the initiation codon of the thromboplastin cDNA to the stop codon of the heavy chain of IgG1. The desired fragment was obtained and, after treatment with HindIII and BamHI, ligated into the vector pCD4E gamma 1, as described above, which had been cut with HindIII (partially) and BamHI. The resulting plasmid was called pTF1Fc (Fig. 4).

#### Transfection of pTF1Fc into mammalian cells

The fusion protein encoded by the plasmid pTF1Fc is called pTF1Fc hereinafter. pTF1Fc was transiently expressed in COS cells. For this purpose, COS cells were

transfected with pTF1Fc with the aid of DEAE-dextran (EP A 0 325 262). Indirect immunofluorescence investigations revealed that the proportion of transfected cells was about 25 %. 24 h after transfection, the cells were transferred into serum-free medium. This cell supernatant was harvested after a further three days.

#### Purification of pTF1Fc fusion protein from cell culture supernatants

170 ml of supernatant from transiently transfected COS cells were collected overnight in a batch process in a column containing 0.8 ml of protein A-Sepharose at 4°C, washed with 10 volumes of washing buffer (50 mM tris buffer pH 8.6, 150 mM NaCl) and eluted in 0.5 ml fractions with eluting buffer (93:7 100 mM citric acid: 100 mM sodium citrate). The first 9 fractions were immediately neutralized with 0.1 ml of 2M tris buffer pH 8.6 in each case and then combined, and the resulting protein was transferred by three concentration/dilution cycles in an Amicon microconcentrator (Centricon 30) into TNE buffer (50 mM tris buffer pH 7.4, 50 mM NaCl, 1 mM EDTA). The pTF1Fc obtained in this way is pure by SDS-PAGE electrophoresis (U.K. Lämmli, Nature 227 (1970) 680-685). In the absence of reducing agents it behaves in the SDS-PAGE like a dimer (about 165 KDa).

#### Biological activity of purified TF1Fc in the prothrombin clotting time determination

TF1Fc fusion protein is active in low concentrations (> 50 ng/ml) in the one-stage prothrombin clotting time determination (Vinazzer, H. Gerinnungsphysiologie und Methoden im Blutgerinnungslabor (1979), Fisher Verlag Stuttgart). The clotting times achieved are comparable with the clotting times obtained with thromboplastin isolated from human placenta.

**Example 2: Interleukin-4 receptor fusion proteins**

Interleukin-4 (IL-4) is synthesized by T cells and was originally called B-cell growth factor because it is able to stimulate B-cell proliferation. It exerts a large number of effects on these cells. One in particular is the stimulation of synthesis of molecules of immunoglobulin subclasses IgG1 and IgE in activated B cells (Coffmann et al., Immunol. Rev., vol. 102 (1988) 5). In addition, IL-4 also regulates the proliferation and differentiation of T cells and other hemopoietic cells. It thus contributes to the regulation of allergic and other immunological reactions. IL-4 binds with high affinity to a specific receptor. The cDNA which codes for the human IL-4 receptor has been isolated (Idzerda et al., J. Exp. Med., vol. 171 (1990) 861-873). It is evident from analysis of the amino-acid sequence deduced from the cDNA sequence that the IL-4 receptor is composed of a total of 825 amino acids, with the 25 N-terminal amino acids acting as signal peptide. Mature human IL-4 receptor is composed of 800 amino acids and, like thromboplastin, has a three-domain structure: i) amino-terminal extracellular domain (207 amino acids); ii) transmembrane region (24 amino acids) and iii) cytoplasmic domain (569 amino acids). In the extracellular domain there are six potential sites for N-glycosylation (Asn-X-Thr/Ser). IL-4 receptor has homologies with human IL-6 receptor, with the  $\beta$ -subunit of human IL-2 receptor, with mouse erythropoietin receptor and with rat prolactin receptor (Idzerda et al., loc. cit.). Thus, like thromboplastin, it is not a member of the immunoglobulin family but is assigned together with the homologous proteins mentioned to the new family of hematopoietin receptors. Members of this family have four cysteine residues and a conserved sequence (Trp-Ser-X-Trp-Ser) in the extracellular domain located near the transmembrane region in common.

On the basis of the described function of the IL-4/IL-4

receptor system, there is a possible therapeutic use of a recombinant form of the IL-4 receptor for suppressing IL-4-mediated immune reactions (for example transplant rejection reaction, autoimmune diseases, allergic reactions).

The amount of substance required for therapy makes it necessary to prepare such molecules by genetic engineering. Because of the straightforward purification by affinity chromatography and improved pharmacokinetic properties, according to the invention the synthesis of soluble forms of the IL-4 receptor as immunoglobulin fusion protein is particularly advantageous.

The IL-4 receptor fusion proteins are secreted by mammalian cells (for example CHO, BHK, COS cells) into the culture medium, purified by affinity chromatography on protein A-Sepharose and have, surprisingly, identical functional properties to the extracellular domain of the intact membrane-bound IL-4 receptor molecule.

Construction of a hybrid plasmid pIL-4R<sub>FC</sub> coding for IL-4 receptor fusion protein.

Cutting of the plasmid pCD4E  $\gamma$ mal1 with XhoI and BamHI results in an opened vector in which the open XhoI site is located downstream from the promoter sequence. The open BamHI site is located at the start of the coding regions for a pentapeptide linker, followed by the hinge and the CH2 and CH3 domains of human IgG1. The reading frame in the BamHI recognition sequence GGATCC is such that GAT is translated as aspartic acid. DNA amplification with thermostable DNA polymerase makes it possible to modify a given sequence in such a way that any desired sequences can be attached at one or both ends. Two oligonucleotides able to hybridize with sequences in the 5'-untranslated region (A: 5' GATCCAGTACTCGAGAGAGAAGCCGGGCGTGGTGGCTCATGC 3') or coding region

(B: 5' CTATGACATGGATCCTGCTCGAAGGGCTCCCTGTAGGAGTTGTG 3') of the IL-4 receptor cDNA which is cloned in the vector pDC302/T22-8 (Idzerda et al., loc. cit.) were synthesized. Of these, oligonucleotide A is partially homologous with the sequence of the coding strand, and oligonucleotide B is partially homologous with the non-coding strand; cf. Fig. 5. Amplification using thermostable DNA polymerase results in a DNA fragment (836 bp) which, based on the coding strand, contains at the 5' end before the start of the coding sequence an XhoI site, and at the 3' end before the last codon of the extracellular domain a BamHI site. The reading frame in the BamHI cleavage site is such that ligation with the BamHI site in pCD4E gamma 1 results in a gene fusion with a reading frame continuous from the initiation codon of the IL-4 receptor cDNA to the stop codon of the heavy chain of IgG1. The desired fragment was obtained and, after treatment with XhoI and BamHI, ligated into the vector pCD4E gamma 1, described above, which had been cut with XhoI/BamHI. The resulting plasmid was called pIL4Rfc (Fig. 6).

#### Transfection of pIL4Rfc into mammalian cells

The fusion protein encoded by the plasmid pIL4Rfc is called pIL4Rfc hereinafter. pIL4Rfc was transiently expressed in COS cells. For this purpose, COS cells were transfected with pIL4Rfc with the aid of DEAE-dextran (EP A 0 325 262). Indirect immunofluorescence investigations revealed that the proportion of transfected cells was about 25 %. 24 h after transfection, the cells were transferred into serum-free medium. This cell supernatant was harvested after a further three days.

#### Purification of IL4Rfc fusion protein from cell culture supernatants

500 ml of supernatant from transiently transfected COS

cells were collected overnight in a batch process in a column containing 1.6 ml of protein A-Sepharose at 4°C, washed with 10 volumes of washing buffer (50 mM tris buffer pH 8.6, 150 mM NaCl) and eluted in 0.5 ml fractions with eluting buffer (93:7 100 mM citric acid: 100 mM sodium citrate). The first 9 fractions were immediately neutralized with 0.1 ml of 2M tris buffer pH 8.6 in each case and then combined, and the resulting protein was transferred by three concentration/dilution cycles in an Amicon microconcentrator (Centricon 30) into TNE buffer (50 mM tris buffer pH 7.4, 50 mM NaCl, 1 mM EDTA). The IL4RFc obtained in this way is pure by SDS-PAGE electrophoresis (U.K. Lämmli, Nature 227 (1970) 680-685). In the absence of reducing agents it behaves in the SDS-PAGE like a dimer (about 150 KDa).

#### Biological activity of purified IL4RFc

IL4RFc proteins binds  $^{125}\text{I}$ -radiolabeled IL-4 with the same affinity ( $K_D=0.5$  nM) as membrane-bound intact IL-4 receptor. It inhibits the proliferation of IL-4-dependent cell line CTLLHuIL-4RI clone D (Idzerda et al., loc. cit.) in concentrations of 10-1000 ng/ml. In addition, it is outstandingly suitable for developing IL-4 binding assays because it can be bound via its Fc part to microtiter plates previously coated with, for example, rabbit anti-human IgG, and in this form likewise binds its ligands with high affinity.

#### Example 3: Erythropoietin fusion proteins

Mature erythropoietin (EPO) is a glycoprotein which is composed of 166 amino acids and is essential for the development of erythrocytes. It stimulates the maturation and the terminal differentiation of erythroid precursor cells. The cDNA for human EPO has been cloned (EP-A-0 267 678) and codes for the 166 amino acids of mature EPO and a signal peptide of 22 amino acids which is essential for secretion. The cDNA can be used to

prepare recombinant functional EPO in genetically manipulated mammalian cells and the EPO can be employed clinically for the therapy of anemic manifestations of various etiologies (for example associated with acute renal failure).

Because of the straightforward purification and the improved pharmacokinetic properties, according to the invention synthesis of EPO as immunoglobulin fusion protein is particularly advantageous.

10      Construction of a hybrid plasmid pEPOFc coding for erythropoietin fusion protein.

This construction was carried out in analogy to that described in Example 2 (section: "Construction of a hybrid plasmid pIL-4RFc coding for IL-4 receptor fusion protein"). Two oligonucleotides able to hybridize with sequences in the vicinity of the initiation codon (A: 5' GATCGATCTCGAGATGGGGTGCACGAATGTCCTGCCTGGCTGTGG 3') and of the stop codon (B: 5' CTGGAATCGGATCCCCTGTCCTGCAGGCCTCCCCTGTGTACAGC 3') of the EPO cDNA cloned in the vector pCES (EP-A 0 267 678) were synthesized. Of these, oligonucleotide A is partially homologous with the sequence of the coding strand, and oligonucleotide B is partially homologous with the non-coding strand; cf. Fig. 7. Amplification with thermostable DNA polymerase results in a DNA fragment (598 bp) which, based on the coding strand, contains at the 5' end in front of the initiation codon an XhoI site and in which at the 3' end the codon for the penultimate C-terminal amino acid residue of the EPO (Asp) is present in a BamHI recognition sequence. The reading frame in the BamHI cleavage site is such that ligation with the BamHI site in pCD4E gamma 1 results in a gene fusion with a reading frame continuous from the initiation codon of EPO cDNA to the stop codon of the heavy chain of IgG1. The desired fragment was obtained and, after treatment with XhoI and BamHI, ligated into

the vector pCD4E gamma 1, described above, which had been cut with XhoI/BamHI. The resulting plasmid was called pEPOFc (Fig. 8).



Patent Claims:

1. A soluble fusion protein composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of immunoglobulin molecules of all subclasses.  
5
2. A fusion protein as claimed in claim 1, wherein the immunoglobulin portion is the constant part of the heavy chain of human IgG.
3. A fusion protein as claimed in claim 2, wherein the immunoglobulin portion is the constant part of the heavy chain of human IgG1 or a protein A-binding fragment thereof.  
10
4. A fusion protein as claimed in claim 2 or claim 3, wherein the fusion takes place at the hinge region.
5. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is the extracellular portion of a membrane protein or parts thereof.  
15
6. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is the extracellular portion of thromboplastin or parts thereof.  
20
7. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is the extracellular portion of a cytokine receptor or growth factor receptor or parts thereof.  
25
8. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of IL-4 receptor or parts thereof.
9. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of IL-7 receptor or parts thereof.  
30

10. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of tumor necrosis factor receptor or parts thereof.
- 5 11. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of G-CSF receptor or parts thereof.
12. A fusion protein as claimed in claim 7, wherein the  
10 portion of GM-CSF receptor or parts thereof.
13. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of erythropoietin receptor or parts thereof.
14. A fusion protein as claimed in claims 1 - 4, wherein  
15 the protein fused to immunoglobulin is a non-membrane-bound soluble protein or part thereof.
15. A fusion protein as claimed in claim 14, wherein the protein fused to immunoglobulin is a cytokine or growth factor or part thereof.
- 20 16. A fusion protein as claimed in claim 15, wherein the protein fused to immunoglobulin is erythropoietin or part thereof.
17. A fusion protein as claimed in claim 15, wherein the  
25 protein fused to immunoglobulin is GM-CSF or G-CSF or part thereof.
18. A fusion protein as claimed in claim 15, wherein the protein fused to immunoglobulin is interleukin IL-1 to IL-8 or part thereof.

19. A fusion protein as claimed in any of preceding claims 1-18, wherein a factor Xa cleavage site is additionally inserted between the immunoglobulin part and the non-immunoglobulin part.
- 5      20. A process for preparing fusion proteins as claimed in any of claims 1 - 19, which comprises introducing the DNA coding for these constructs into a mammalian cell expression system and, after expression, purifying the produced fusion protein by affinity chromatography via the immunoglobulin portion.
- 10
21. The use of the fusion proteins as claimed in any of claims 1 - 19 for diagnosis.
22. The use of the fusion proteins as claimed in any of claims 1 - 19 for therapy.
23. A fusion protein as claimed in claim 1, substantially as hereinbefore described and exemplified.
24. A process for preparing a fusion protein as claimed in claim 1, substantially as hereinbefore described and exemplified.
25. A fusion protein as claimed in claim 1, whenever prepared by a process claimed in claim 20 or 24.

Dated this the 27th day of June, 1991

F. R. KELLY & CO.

BY:  EXECUTIVE

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AGENTS FOR THE APPLICANTS

***Fig. 1***

121 GTCGCTCGGACGCTCCTGCTCGGCTGGGTCTTCGCCCAGGTGGCCGGCGCTTCAGGCACT  
 -----+-----+-----+-----+-----+ 180  
 CAGCGAGCCTGCGAGGACGAGCCGACCCAGAAGCGGGTCCACCGCCGCGAAGTCCGTGA  
 <\*\*\*\*\*  
 Oligonucleotide 1

181 ACAAATACTGTGGCAGCATATAATTTAACTTGGAAATCAACTAATTTCAAGACAATTTTG  
 -----+-----+-----+-----+-----+ 240  
 TGTATTATGACACCGTCGTATATTAATTGAACCTTTAGTTGATTAAAGTTCTGTAAAAAC  
 \*\*\*\*\*|

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Oligonucleotide 2  
 |\*\*\*\*\*>  
 721 AACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCCCGAACAGTTAACCGGAAGAGTACA  
 -----+-----+-----+-----+-----+ 780  
 TTGATGACAAAGTCACAAGTTCGTCACTAAGGGAGGGCTTGTCAATTGGCCTTCTCATGT

***Fig. 2***

10 30 50  
GCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTCTCGGCGAACCCC

70 90 110  
CTCGCACTCCCTCTGGCCGGCCCAGGGCGCCTTCAGCCCCAACCTCCCCAGCCCCACGGGC

130 150 170  
GCCACGGAACCCGCTCGATCTCGCCGCCAACTGGTAGACATGGAGACCCCTGCCTGGCCC  
MetGluThrProAlaTrpPro

190 210 230  
CGGGTCCCGCGCCCCGAGACCGCCGTCGCTCGGACGCTCCTGCTCGGCTGGGTCTTCGCC  
ArgValProArgProGluThrAlaValAlaArgThrLeuLeuLeuGlyTrpValPheAla

250 270 290  
CAGGTGGCCGGCGCTTCAGGCACTACAAATACTGTGGCAGCATATAATTTAACTTGAAAA  
GlnValAlaGlyAlaSerGlyThrThrAsnThrValAlaAlaTyrAsnLeuThrTrpLys

310 330 350  
TCAACTAATTTCAAGACAATTTTGGAGTGGGAACCCAAACCCGTCAATCAAGTCTACACT  
SerThrAsnPheLysThrIleLeuGluTrpGluProLysProValAsnGlnValTyrThr

370 390 410  
GTTCAAATAAGCACTAAGTCAGGAGATTGGAAAAGCAAATGCTTTTACACAACAGACACA  
ValGlnIleSerThrLysSerGlyAspTrpLysSerLysCysPheTyrThrThrAspThr

430 450 470  
GAGTGTGACCTCACCGACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTC  
GluCysAspLeuThrAspGluIleValLysAspValLysGlnThrTyrLeuAlaArgVal

490 510 530  
TTCTCCTACCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCTGTATGAG  
PheSerTyrProAlaGlyAsnValGluSerThrGlySerAlaGlyGluProLeuTyrGlu

550 570 590  
AACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAGCCAACAATTCAGAGT  
AsnSerProGluPheThrProTyrLeuGluThrAsnLeuGlyGlnProThrIleGlnSer

***Fig. 2 (cont.)***

610 630 650  
TTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAGAAGATGAACGGACTTTAGTCAGA  
PheGluGlnValGlyThrLysValAsnValThrValGluAspGluArgThrLeuValArg

670 690 710  
AGGAACAACACTTTCTAAGCCTCCGGGATGTTTTTGGCAAGGACTTAATTTATACACTT  
ArgAsnAsnThrPheLeuSerLeuArgAspValPheGlyLysAspLeuIleTyrThrLeu

730 750 770  
TATTATTGGAAATCTTCAAGTTCAGGAAAGAAAACAGCCAAAACAACTAATGAGTTT  
TyrTyrTrpLysSerSerSerSerGlyLysLysThrAlaLysThrAsnThrAsnGluPhe

790 810 830  
TTGATTGATGTGGATAAAGGAGAAACTACTGTTTCAGTGTTCAGCAGTGATTCCCTCC  
LeuIleAspValAspLysGlyGluAsnTyrCysPheSerValGlnAlaValIleProSer

850 870 890  
CGAACAGTTAACCGGAAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAGGG  
ArgThrValAsnArgLysSerThrAspSerProValGluCysMetGlyGlnGluLysGly

910 930 950  
GAATTCAGAGAAATATTCTACATCATTGGAGCTGTGGTATTTGTGGTCATCATCCTTGTC  
GluPheArgGluIlePheTyrIleIleGlyAlaValValPheValValIleIleLeuVal

970 990 1010  
ATCATCCTGGCTATATCTCTACACAAGTGTAGAAAGGCAGGAGTGGGGCAGAGCTGGAAG  
IleIleLeuAlaIleSerLeuHisLysCysArgLysAlaGlyValGlyGlnSerTrpLys

1030 1050 1070  
GAGAACTCCCCACTGAATGTTTCATAAAGGAAGCACTGTTGGAGCTACTGCAAATGCTAT  
GluAsnSerProLeuAsnValSer

1090 1110 1130  
ATTGCACTGTGACCGAGAACTTTTAAGAGGATAGAATACATGGAAACGCAAATGAGTATT

1150 1170 1190  
TCGGAGCATGAAGACCCTGGAGTTCAAAAACTCTTGATATGACCTGTTATTACCATTAG

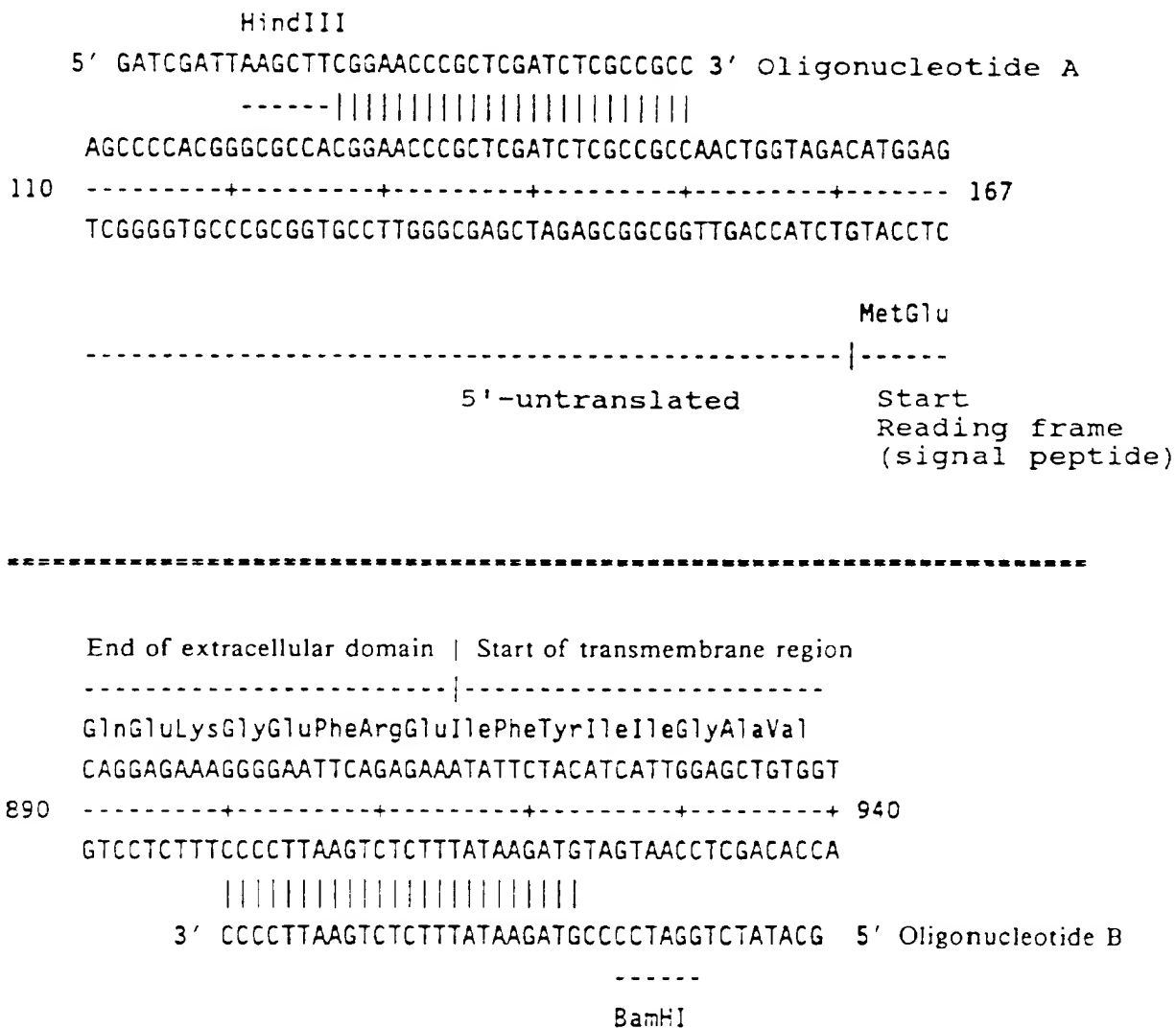
***Fig. 2 (cont.)***

1210	1230	1250
CATTCTGGTTTTGACATCAGCATTAGTCACTTTGAAATGTAACGAATGGTACTACAACCA		
1270	1290	1310
ATTCCAAGTTTTAATTTTTAACACCATGGCACCTTTTGCACATAACATGCTTTAGATTAT		
1330	1350	1370
ATATTCGGCACTTAAGGATTAACCAGGTCGTCCAAGCAAAAACAAATGGGAAAATGTCTT		
1390	1410	1430
AAAAAATCCTGGGTGGACTTTTGAAAAGCTTTTTTTTTTTTTTTTTTTTTTTTGAGACGGAGTC		
1450	1470	1490
TTGCTCTGTTGCCAGGCTGGAGTGCAGTAGCACGATCTCGGCTCACTTGCACCCTCCGT		
1510	1530	1550
CTCTCGGGTTCAAGCAATTGTCTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGTGCGC		
1570	1590	1610
ACTACCACGCCAAGCTAATTTTTGTATTTTTTAGTAGAGATGGGGTTTCACCATCTTGGC		
1630	1650	1670
CAGGCTGGTCTTGAATTCCTGACCTCAGTGATCCACCCACCTTGGCCTCCCAAAGATGCT		
1690	1710	1730
AGTATTATGGGCGTGAACCACCATGCCCAGCCGAAAAGCTTTTGAGGGGCTGACTTCAAT		
1750	1770	1790
CCATGTAGGAAAGTAAAATGGAAGGAAATTGGGTGCATTTCTAGGACTTTTCTAACATAT		
1810	1830	1850
GTCTATAATATAGTGTTTAGGTTCTTTTTTTTTTTCAGGAATACATTTGGAAATTCAAAAC		
1870	1890	1910
AATTGGGCAAACTTTGTATTAATGTGTTAAGTGCAGGAGACATTGGTATTCTGGGCAGCT		

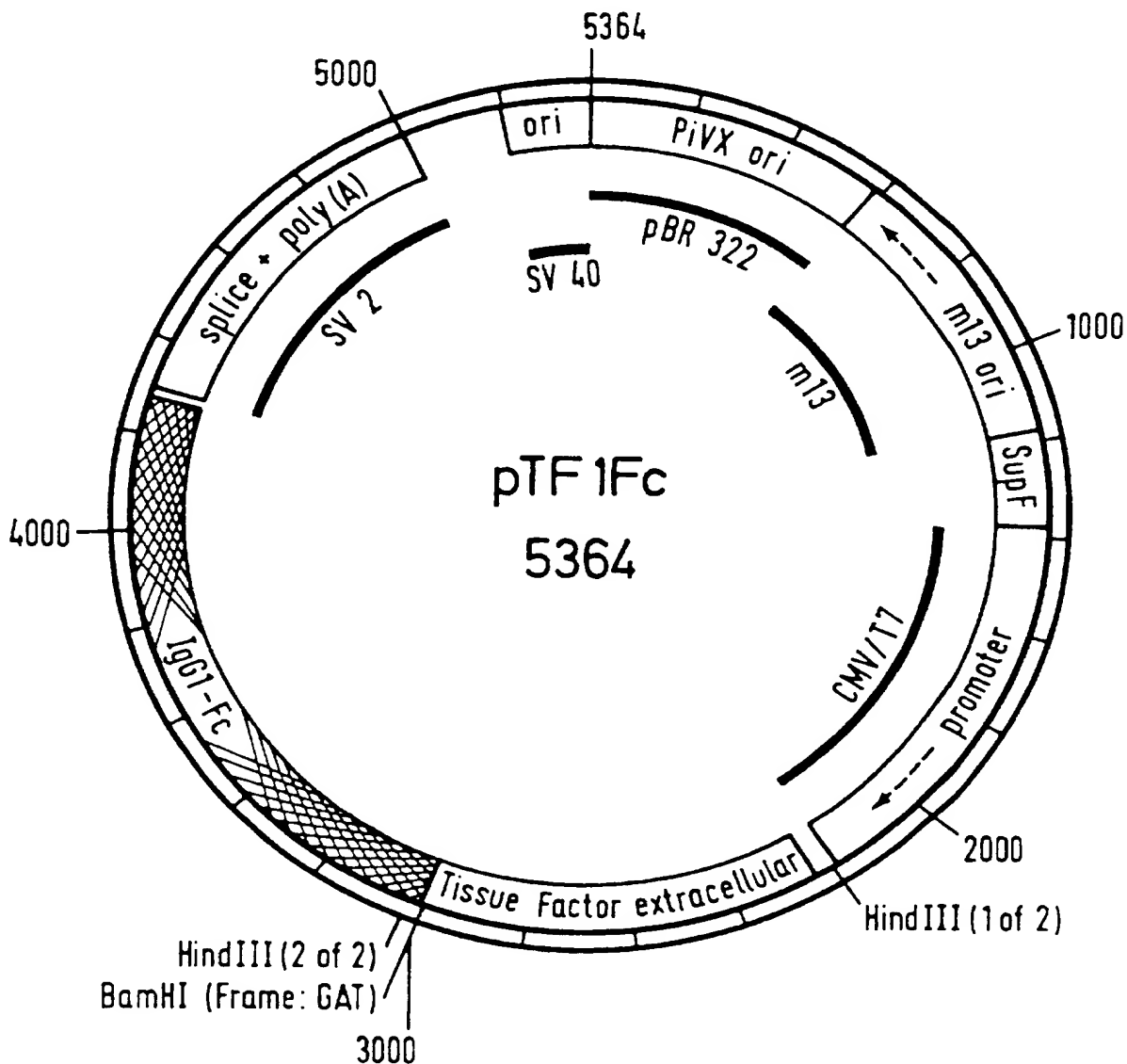
***Fig. 2 (cont.)***

1930	1950	1970
TCCTAATATGCTTTACAATCTGCACTTTAACTGACTTAAGTGGCATTAAACATTTGAGAG		
1990	2010	2030
CTAACTATATTTTTATAAGACTACTATACAACTACAGAGTTTATGATTTAAGGTACTTA		
2050	2070	2090
AAGCTTCTATGGTTGACATTGTATATATAATTTTTTAAAAAGGTTTTTCTATATGGGGAT		
2110	2130	2150
TTTCTATTTATGTAGGTAATATTGTTCTATTTGTATATATTGAGATAATTTATTTAATAT		
2170		
ACTTTAAATAAAGGTGACTGGGAATTGTT		



***Fig. 3***

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**Fig. 4**

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**Fig. 5**

Xho I

5' GATCCAGTACTCGAGAGAGAAGCCGGGCGTGGTGGCTCATGC 3' oligonucleotide A

[illegible]

-----5'-untranslated-----

GCAGATCACTTGAGATCAGGAGTTCGAGACCAGCCTGGTGCCCTGGCATCTCCCAATGGG  
121 -----+-----+-----+-----+-----+-----+ 180  
CGTCTAGTGAACCTCTAGTCCTCAAGCTCTGGTCGGACCACGGAACCGTAGAGGGTTACCC

-----5'-untranslated-----|MetGly

Reading frame (signal peptide) Start

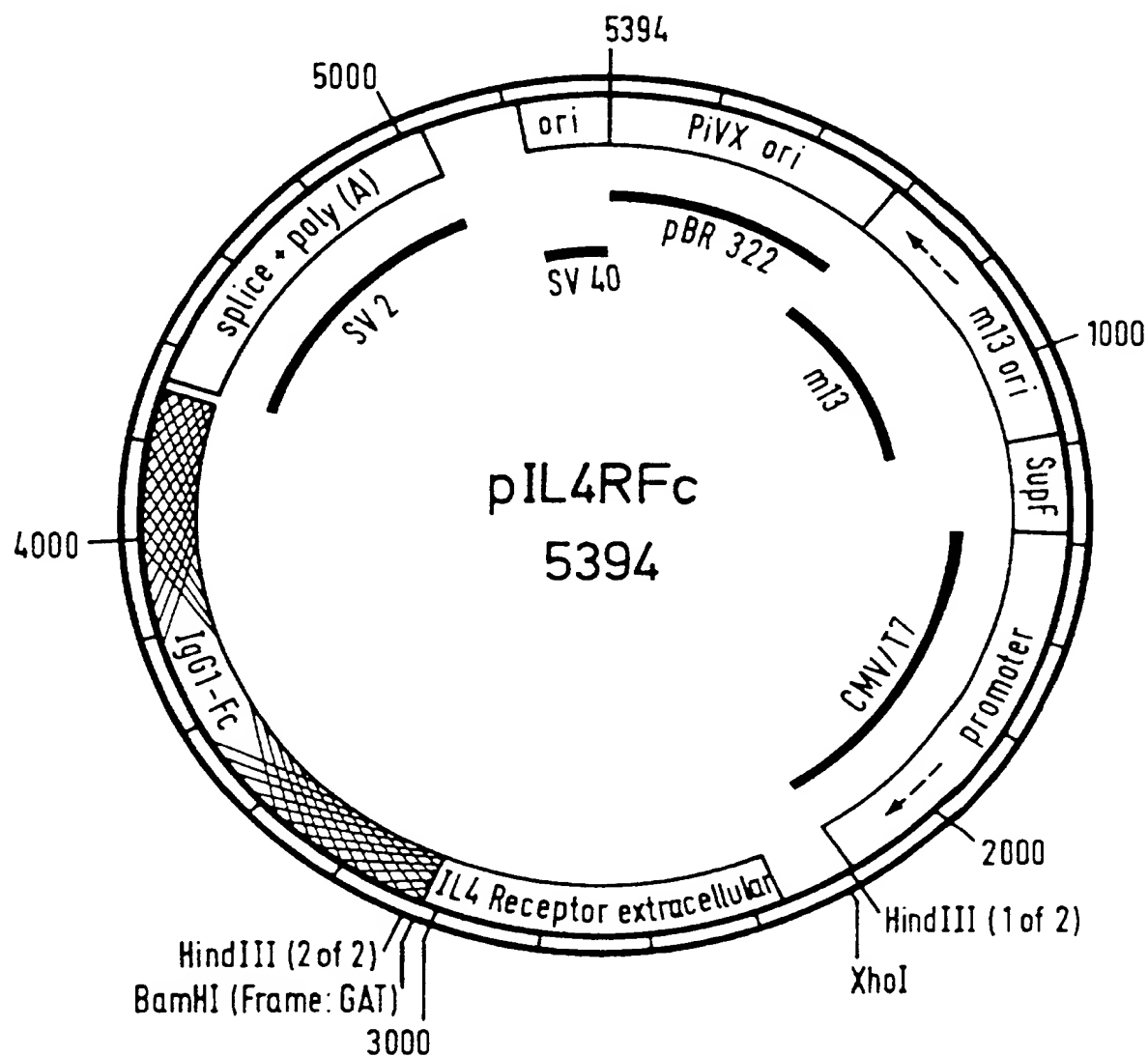
00 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038

End of extracellular domain | Start of transmembrane region

-----|-----  
HisAsnSerTyrArgGluProPheGluGlnHisLeuLeuLeuGlyValSerValSerCys  
CACAACTCCTACAGGGAGCCCTTCGAGCAGCACCTCTGCTGGGGGTCAAGCGTTTCCTGC  
839 ++++++ 898  
GTGTTGAGGATGTCCCTCGGGAAGCTCGTCGTGGAGGACGACC CGCAGTCGCAAAGGACG

3' GTGTTGAGGATGTCCCTCGGGAAGCTCGTCTTAGGTACAGTATC 5' Oligonucleotide B

Баш:



**Fig. 6**

***Fig. 1***

XhoI

5' GATCGATCTCGAGATGGGGGTGCACGAATGTCCTGCCTGGCTGTGG 3' Oligonucleotide A

-----|

ATGGGGGTGCACGAATGTCCTGCCTGGCTGTGGCTTCTCCTGTCCCTGCTGTGG

182 -----+-----+-----+-----+-----+----- 235

TACCCCCACGTGCTTACAGGACGGACCGACACCGAAGAGGACAGGGACGACAGC

MetGlyValHisGluCysProAlaTrpLeuTrpLeuLeuSerLeuLeuSer -

Start reading frame (signal peptide)

-----

End of reading frame-----|

LeuTyrThrGlyGluAlaCysArgThrGlyAspArgEnd

-----|

GCTGTACACAGGGGAGGCCTGCAGGACAGGGGACAGATGACCAGGTGTGTCCACCTGGGC

724 -----+-----+-----+-----+-----+----- 783

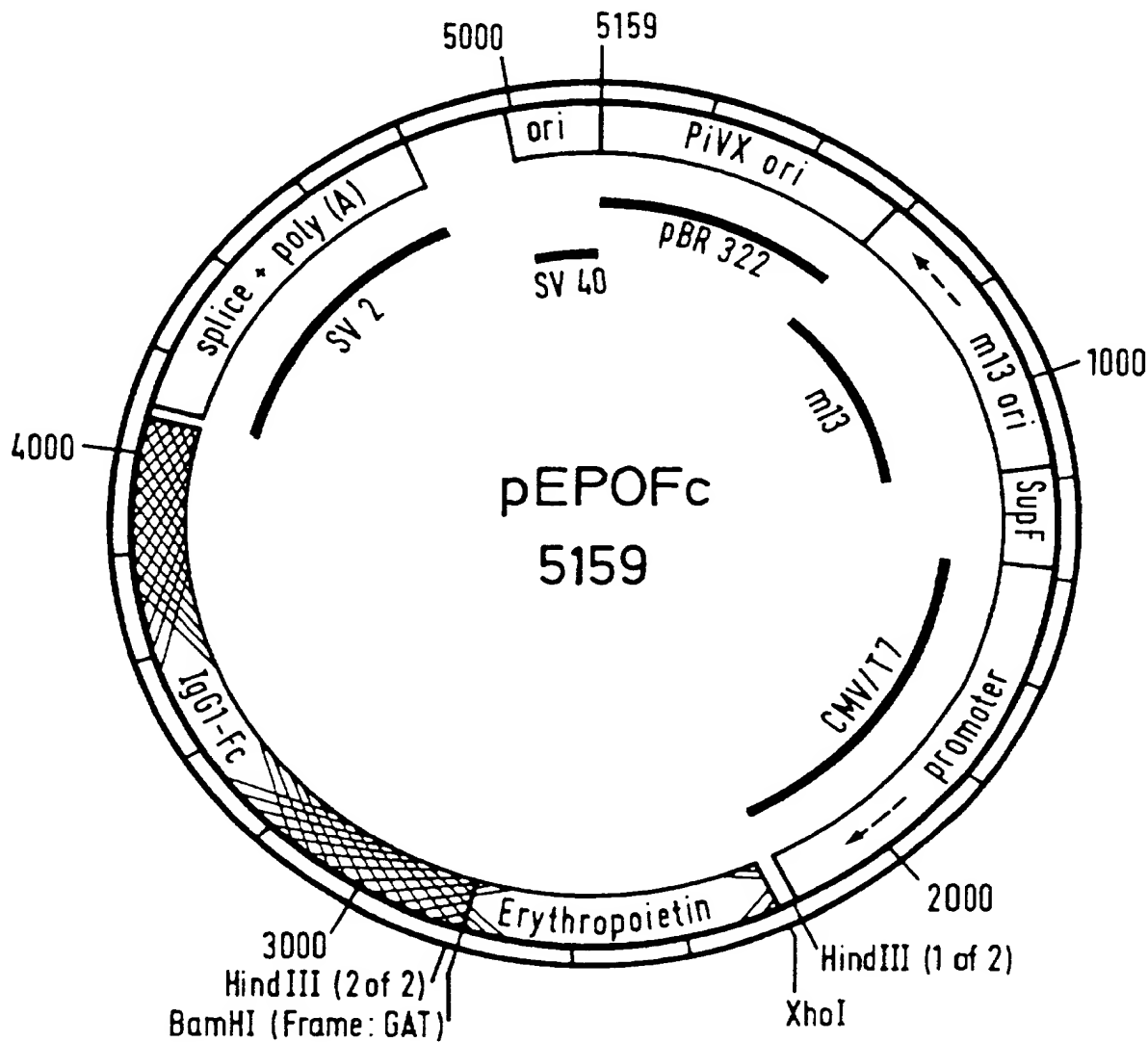
CGACATGTGTCCCTCCGGACGTCCTGTCCCTGTCTACTGGTCCACACAGGTGGACCCG

|

3' CGACATGTGTCCCTCCGGACGTCCTGTCCCTAGGCTAAGGTC 5' Oligonucleotide B

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BamHI



**Fig. 8**